Journal of Surgical Oncology 64:289–294 (1997)

Gene Therapy for Colon Cancer With the Herpes Simplex Thymidine Kinase Gene

CHARLES J. LINK, JR.,[1]* JOHN P. LEVY,[1] LEISA Z. McCANN,[1] AND DONALD W. MOORMAN[2]
[1]*Human Gene Therapy Research Institute, Central Iowa Health Systems, Des Moines, Iowa*
[2]*From the Department of Surgery Education, Iowa Methodist Medical Center, Des Moines, Iowa*

Background: The suicide gene and prodrug, herpes simplex thymidine kinase (HStk) and ganciclovir (GCV), are now in clinical trials for recurrent malignancies.
Methods: We evaluated in vitro and in vivo efficacy of HStk gene transfer and GCV treatment of colonic adenocarcinoma in a syngeneic murine model.
Results: In vitro analysis demonstrated that CT-26 adenocarcinoma cells transduced with LTKOSN.2 retroviral vector inhibited the proliferation of wild-type CT-26 (nontransduced) cells after GCV exposure. Cooperative killing with HStk gene therapy was shown in vivo, mixtures of HStk CT-26 transduced cells (CT-26 TK), and nontransduced (CT-26 NV) cells and tumors containing only 9% CT-26 TK cells demonstrated complete regression after GCV (100 mg/kg).
Conclusions: This in vitro and in vivo demonstration suggests that metabolic cooperation permits destruction of tumors even when gene transfer is effective only to a relatively small portion of the tumor. These important results suggest new avenues can be developed for the treatment of this lethal malignancy. *J. Surg. Oncol. 64:289–294, 1997*

KEY WORDS: **CT-26 colonic adenocarcinoma; ganciclovir; gene therapy; retrovirus; vector producer cell; herpes simplex thymidine kinase gene**

INTRODUCTION

Suicide gene therapy strategies aim to insert a new gene to produce an enzyme that catalyzes the conversion of a nontoxic or minimally toxic prodrug to a toxic form within the tumor cell [1]. In essence, cells that receive the new enzymatic function become sensitized to a previously nontoxic drug. The selectivity of the antitumor effect is related to the degree of specific gene transfer or vector gene expression within tumor cells. The herpes simplex thymidine kinase (HStk) gene is a negative selectable marker that can be used as a suicide gene. HStk sensitizes the transduced tumor cells to ganciclovir (GCV), an antiviral drug [1]. GCV is a substrate for phosphorylation by HStk, resulting in a monophosphate (MP) form of the drug (Fig. 1). Cellular phosphorylases convert this GCV-MP to GCV-triphosphate (GCV-TP), which inhibits DNA polymerase and incorporates into DNA. The result is cell death for the HStk-expressing cells [2–5]. The human thymidine kinase enzyme has very low affinity for GCV, so little systemic toxicity is observed in normal tissues. Retrovirus-mediated gene transfer experiments using the HStk gene are now in clinical trials for brain and ovarian malignancies [6,7].

Three general approaches are used to target gene therapy. First, retroviral vectors producer cells, producing retroviral vectors that only integrate into dividing cells, allow for selective targeting of genes into proliferating tumor over normal quiescent tissues in the injection area [7–10]. Second, gene expression is made conditionally dependent on the activity of a tumor-specific pro-

Abbreviations: GCV, ganciclovir; VPC, vector producer cell; HStk, herpes simplex thymidine kinase

*Correspondence to: Dr. C. Link, Jr., Gene Therapy Program, Human Gene Therapy Research Institute, 1415 Woodland Ave., Des Moines, IA 50325; E-mail: hgtriii.netins.net

Accepted 6 January 1997

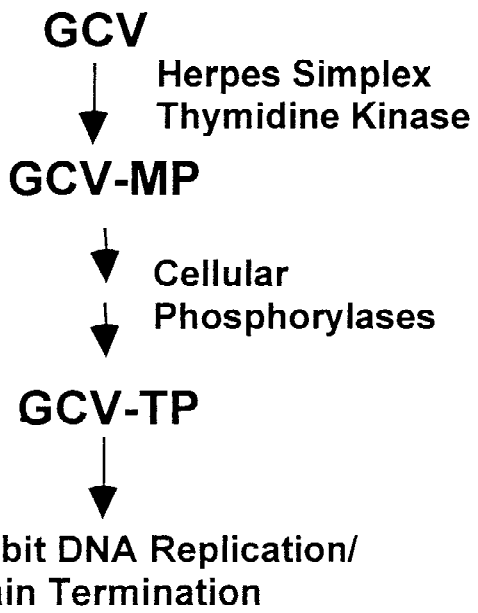

Fig. 1. Metabolic pathway of ganciclovir. Herpes simplex thymidine kinase (HStk) activation of ganciclovir (GCV) to GCV-monophosphate (GCV-MP). Cellular phosphorylases then convert GCV-MP to GCV-triphosphate (GCV-TP). Cells undergoing mitosis are then inhibited by the drug.

moter (e.g., melanoma) [11]. Thus, even if gene delivery is not tumor specific, the toxic gene would only be expressed in tumor cells. Finally, since the killing effect of some drugs is dependent on DNA synthesis, nondividing tissues are resistant to activated drug effects, even if gene expression occurs [12].

The HStk/GCV system is active in animal models in vitro and in vivo. The subcutaneous injection of HStk-transfected cells was followed by GCV treatment once an established tumor had formed [5]. These HStk-containing tumors were routinely destroyed with this technique. Mixtures of HStk-positive and HStk-negative cells responded in vitro to GCV, confirming the presence of a bystander effect between the cells [9,13–15]. This method of tumor destruction does not seem to involve generalized nonspecific cellular toxicity. Skin and other tissues overlying HStk-treated tumors are not injured, while the tumors expressing the HStk gene and the admixed wild-type tumor cells are completely destroyed [7,16,17].

Our experiments evaluated both the in vitro and in vivo value of HStk gene transfer (ex vivo) with tumor cell inhibition or destruction. Our studies confirmed that HStk gene-containing retroviral vectors can effectively transduce CT-26 tumor cells and express the HStk gene, resulting in 100% kill of partially transduced, macroscopic tumors in BALB/c mice treated with GCV. We also found that BALB/c mice were more sensitive to toxic side effects of GCV, than C57BI/6 mice or athymic nude mice.

MATERIALS AND METHODS

Construction of the pLTKOSN Retroviral Vector

The vector was constructed from LXSN, which contains safety modifications to reduce the chances of retroviral vector recombination, leading to the regeneration of replication-competent virus [18]. The retroviral backbone includes a NeoR selectable gene encoding neomycin phosphotransferase II (NPT II), which protects cells from the toxic effects of G418, a neomycin analog. The pLTKOSN vector was constructed by polymerase chain reaction (PCR) amplifying the 1179-bp HStk open reading frame (ORF) of the HStk gene into the *Eco*RI site of pLXSN. The HStk ORF was amplified with the upstream primer 5′CCA AGC TTC GGC CAG CGC CTA 3′ (25mer) and downstream primer 5′CCA AGC TTC CGG TAT TGT CTC CTT CC3′ (26mer). This was cloned into pCRII (Invitrogen, Carlsbad, CA) which provided Eco RI sites that were then used to subclone into the multicloning site (*Eco*RI) of LXSN.

Cell Culture

CT-26 colon adenocarcinoma cells (American Type Tissue Culture Collection, Rockville, MD) are syngeneic with BALB/c mice. EMT-6 is a murine breast adenocarcinoma cell line (ATCC, Rockville, MD) syngeneic with BALB/c mice. MCA 205 murine fibrosarcoma cells (kindly provided by Dr. S. Rosenberg, NCI, Bethesda, MD) is syngeneic with C57B1/6 mice. All mice were obtained from Harlan Sprague Dawley (Indianapolis, IN). The PA317 cell line was also obtained from ATCC. The tumor cells were grown in D10 Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) (Gibco BRL) in monolayers at 37°C and 5% CO_2. Vector producer cells (VPC) were grown in DMEM in monolayers at 37°C and 5% CO_2. Cells were passaged at 80–90% confluence and harvested by standard trypsin (Gibco BRL, Grand Island, NY) digestion at 37°C.

Construction of the LTKOSN.2 Vector Producer Cell Line

The plasmid pLTKOSN was transfected into the amphotropic packaging cell line PA317 and selected in 1 mg/ml G418. After selection, the transfected pLTKOSN/PA317 VPC line was used to transduce new PA317 cells. Supernate was collected from a culture of pLTKOSN/PA317 VPC that was 90–100% confluent. Supernate was filtered through 0.22-μm filters (Nalgene, Rochester, NY), supplemented with 2 μg/ml of protamine sulfate (Fujisawa, USA, Deerfield, IL) and transferred into tissue culture flasks containing PA317 cells. The PA317 cells were transduced twice daily for 3 days. The resulting vector transduced cells were designated LTKOSN/PA317 VPC. At 24 hr after the final exposure to retro-

viral supernate, the cells were placed in G418 (1 mg/ml) selection for 2 weeks. These cells were subsequently subcloned and the clone, designated LTKOSN.2 VPC, was grown in quantity for use in the following experiments.

Transduction of Tumor Cells With the LTKOSN.2 Retroviral Vector

The LTKOSN.2 VPC were used to transduce CT-26 and EMT-6 tumor cells. Supernate was collected from cultures of LTKOSN.2 VPC when they were 90–100% confluent. LTKOSN.2 VPC have a titer of 1.6×10^6 cfu/ml. Supernate was filtered through 0.22-μm filters (Nalgene), supplemented with 2 μg/ml of protamine sulfate (Fujisawa, USA, Deerfield, IL) and transferred into tissue culture flasks containing tumor cells that were in log phase growth. The target cells were transduced twice daily for 3 days. At 24 hr after the final exposure to retroviral supernate the cells were selected in G418 (1 mg/ml) for 2 weeks. The surviving HStk transduced cells were then pooled for in vitro and in vivo experiments.

In Vitro Cooperative Inhibition Studies of LTKOSN.2 Tumor Cells on Nontransduced Tumor Cells

In vitro HStk proliferation assays were performed to characterize further the nature of the potentiation of GCV toxicity between LTKOSN.2 transduced CT-26 cells (CT-26 TK) and CT-26 cells with no vector (CT-26 NV). Cell mixtures of CT-26 TK and/or CT-26 NV cells were plated. CT-26 colonic adenocarcinoma cells were harvested from 80–90% confluent cultures by trypsin digest, counted and plated at 10^4 cells/well in 200-μl DMEM with 10% FCS media (Gibco, BRL) in 96 well flat-bottom plates (Costar, Cambridge, MA). The percent of nontransduced tumor cells (CT-26 NV) varied from 100% to 0%. Three replicate sample mixtures were plated for each mixture. The cells were incubated overnight at 37°C in DMEM supplemented with 10% fetal calf serum. GCV (Syntex, Palo Alto, CA) was then added at concentrations of 0 or 5.1 μg/ml. Forty-four hr later 0.5 μCi ^{3}(H)-thymidine (New England Nuclear, Boston, MA) was added to each well and the cells were incubated at 37°C for an additional 4 hr. Cells were then trypsin digested for 15–20 min at 37°C, then the plates were washed and transferred to filter mats in a Tomtec (Orange, CT) cell harvester. Filter mats were then counted. The amount of incorporated ^{3}H-thymidine is measured as counts per minute (cpm). Each condition was cultured in triplicate wells and the values expressed as a percentage of control (no GCV).

Toxicity of Ganciclovir in Athymic Nude, C57BI/6, and BALB/c Mice

Mice were included from each of three strains of mice (athymic nude [nu/nu], C57BI/6, and BALB/c). Three mice of each strain were injected with either 50, 75, 100, 125, or 150 mg/kg of GCV. GCV was administered in 1.0 ml of Hank's balanced salt solution (HBSS) twice daily for 14 days. The concentration of GCV ranged from 1.0 to 3.0 mg/ml and was administered as an intraperitoneal injection. In addition to the toxicity analysis, three animals of each group were injected with 4×10^6 LTKOSN.2 transduced cells and then treated with 100 mg/kg GCV dosage twice daily for 14 days. Three athymic nude mice were injected with 4×10^6 LTKOSN.2 VPC. Three BALB/c mice were injected with EMT-6 breast carcinoma cells transduced with the LTKOSN.2 vector. Three C57BI/6 mice were injected with MCA 205 fibrosarcoma cells transduced with the LTKOSN.2 vector. Animals were then followed for antitumor responses.

In Vivo Evaluation of Antitumor Effect from the HStk Gene and Ganciclovir in Subcutaneous CT-26 Colon Carcinoma

We evaluated the ability of transduced CT-26 tumor cells to cause the regression of nontransduced CT-26 tumor cells. Six- to 8-week-old BALB/c mice were obtained from Harlan Sprague-Dawley (Indianapolis, IN). The animals were separated into groups of five mice per standard cage. The animals were fed and watered ad libitum. One cage was used for each treatment condition. Briefly, 1×10^5 nontransduced CT-26 cells (CT-26 NV) were mixed with 0%, 9%, or 50% HStk-transduced CT-26 cells (CT-26 TK) and were suspended in 100 μl HBSS (Gibco, BRL) and injected subcutaneously into the anterior abdominal wall of the animals on day one of the experiment. Control animals received 1×10^5 CT-26 NV cells only. Five days later, the animals received a 14-day course of ganciclovir (days 6–19) at a dose of 150 mg/kg IP bid (1 ml of 3 mg/ml of HBSS). Serial measurements of tumor volume in three dimensions (mm) were recorded.

RESULTS

In Vitro GCV Sensitivity of CT-26 Adenocarcinoma Cells After Transduction With a HStk Vector

Figure 2 plots data obtained from an experiment investigating the in vitro bystander effect of CT-26 transduced by LTKOSN.2 VPC supernate on nontransduced CT-26 cells. CT-26 tumor cells were transduced in vitro using filtered supernate from confluent LTKOSN.2 VPC. After G418 selection, only cells expressing a functional NeoR gene are able to survive, producing an essentially 100% selected population of vector transduced cells. We evaluated the sensitivity of the retroviral vector, transduced, G418-selected cells compared to the nontransduced parent cell lines to GCV or mixtures of HStk-transduced and -nontransduced cells. GCV concentrations of 5 μg/ml was inhibitory to HStk-transduced CT-26 cell lines in this assay.

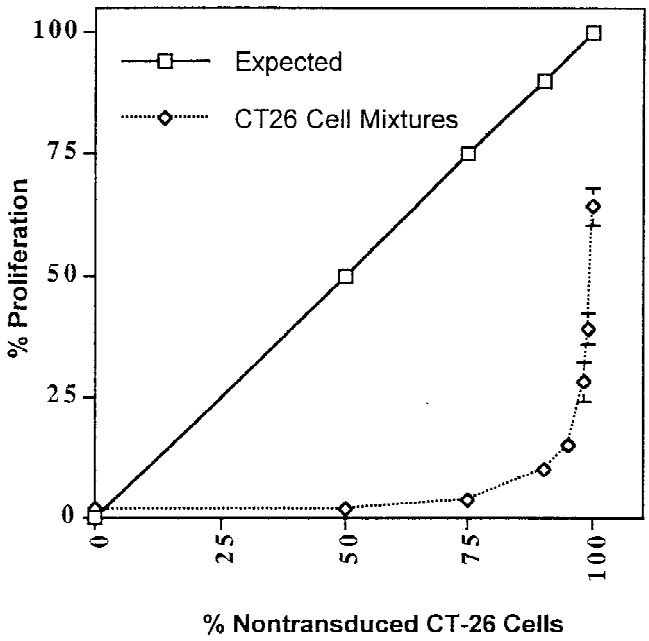

Fig. 2. In vitro bystander effect of CT-26 cells transduced with LTKOSN.2 on CT-26 NV cells. Either HStk-transduced (CT-26 TK) or nontransduced (CT-26 NV) tumor cells were plated in 96-well microtiter plates. Mixtures of a total of 10,000 tumor cells with varying proportions of CT-26 TK and CT-26 NV cells with plated. GCV was then added to the wells at a concentration of 5 μg/ml; 44 hr later, the cultures were pulsed with tritiated thymidine. At 4 hr later, the cultures were harvested. The data were collected as counts per minute (cpm), and the mean of triplicate wells has been presented. The expected curve represents the theoretical percentage of proliferation inhibition if only the HStk-transducted cells are inhibited.

Mixtures of cells containing differing ratios of CT-26 cells transduced with LTKOSN.2 and nontransduced CT-26 cells (1×10^4) were plated in flat bottom, 96-well plates and treated with 5 μg/ml GCV and then labeled with ^{3}H-thymidine (described in Procedures and Methods Section). In this experiment, the ''expected'' kill curve would predict that combinations containing 50% TK-transduced tumor cells and 50% NV cells would be inhibited by 50%. The 5 μg/ml GCV concentration is within the therapeutic range established in humans with a dose of 5 mg/kg/dose. Since it is unlikely that 100% of the tumor cells will be successfully gene modified, this cooperative inhibitory effect is a crucial component for the success of this approach.

Ganciclovir Toxicity in Various Mouse Strains

In order to determine the toxicity from GCV in our murine tumor models, we conducted a dose escalation study in athymic nude (nu/nu), BALB/c, and C57BI/6 mouse strains. The results of the toxicity study are summarized in Table 1. Generally, C57BI/6 mice tolerated the GCV without significant toxicity. BALB/c mice that received more than 100 mg/kg all died from GCV toxicity either during or shortly after completion of therapy.

TABLE I. Ganciclovir Toxicity in Different Strains of Mice*

GCV dose (mg/kg bid)	Mouse strain (surviving mice/total mice)		
	BALB/c	C57BI/6	nu/nu
50	3/3	3/3	3/3
75	3/3	3/3	3/3
100	3/3	3/3	3/3
125	1/3	3/3	3/3
150	0/3	3/3	3/3

*Three animals were treated in each group. Ganciclovir was administered twice daily by intraperitoneal injection for 14 days at the dose indicated.

Athymic nude mice demonstrated an intermediate level of toxicity from the GCV. Although no animals died from GCV, some did become less active and slightly cachectic from the two highest drug doses.

Controls were also conducted to determine whether the 100 mg/kg GCV dosage was sufficient to destroy HStk-transduced cells. Three athymic nude mice, three BALB/c mice, and three C57BI/6 mice, were injected subcutaneously with 4×10^6 LTKOSN.2 VPC, LTKOSN.2 EMT-6 cells, or LTKOSN.2 MCA 205 cells, respectively. Prior experiments (Link, unpublished observations) demonstrated that this number of cells would result in progressive tumor growth in the appropriate mouse strain. All the tumors that initially formed in these mice resolved completely after GCV treatment. Some small tumors did recur in some animals after resolution of GCV treatments.

In Vivo Antitumor Efficacy of HStk Gene and Ganciclovir in Subcutaneous CT-26 Colon Carcinoma

To investigate the effectiveness of HStk gene therapy in vivo, mixtures of CT-26 TK and CT-26 NV cells were injected subcutaneously into BALB/c mice. Tumors grew on the anterior abdominal wall in all animals injected subcutaneously with mixtures of CT-26 NV and/or CT-26 TK in 100 μl of HBSS. GCV was administered twice daily for 14 days. The data for tumor growth are depicted graphically in Figure 3. Animals injected with 100% CT-26 TK cells that did not receive GCV had progressive tumor growth. Animals with tumors containing 100% CT-26 NV cells did not respond to therapy with GCV. However, animals containing either 100% CT-26 TK cells or a 1:10 mixure of CT-26 TK to CT-26 NV cells had excellent responses (Table II). The animals with tumors containing 50% CT-26 TK cells demonstrated complete regression after GCV (100 mg/kg). However, tumors containing 9% CT-26 TK cells showed substantial decrease in growth but not complete responses (Table II). These results demonstrate a very effective in vivo cooperative killing effect of the LTKOSN.2 transduced cells on nontransduced cells.

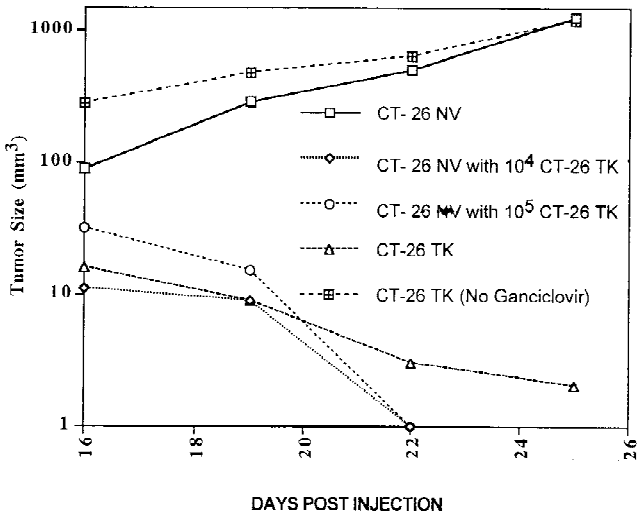

Fig. 3. In vivo mixing experiment with transduced and nontransduced CT-26 tumor cells. CT-26 tumor cells were injected subcutaneously and allowed to grow for up to 26 days. Mice received mixtures containing either 10^5 nontransduced CT-26 tumor cells (CT-26 NV); 10^5 nontransduced CT-26 tumor cells mixed with 10^5 HStk-transduced cell (CT-26 NV with 10^5 CT-26 TK), 10^5 nontransduced CT-26 tumor cells mixed with 10^4 HStk-transduced cell (CT-26 NV with 10^5 CT-26 TK), 10^5 HStk-transduced cells (CT-26 TK) with GCV, or 10^5 HStk-transduced CT-26 cells without drug. Mice with tumors containing 100% CT-26 NV cells grew at a rapid rate despite GCV treatment. Mice with tumors containing 100% CT-26 TK cells not treated with GCV also grew rapidly. Mice injected with either 100% CT-26 TK cells or mixtures containing CT-26 TK cells had excellent antitumor responses after GCV administration.

DISCUSSION

A number of investigators are following the lead provided by F. Moolten in developing the HStk gene for the treatment of solid tumors [1,5]. Recently published work suggests the feasibility of HStk gene transfer for the treatment of solid tumors. The antitumor effect of HStk VPC implantation was tested in growing 9L gliosarcoma in rats in an effort to establish a model for human glioblastoma multiforme, although GCV selection may not be required in this model [9,19,20]. These findings supported the development of a human clinical trial of HStk gene therapy [6]. Evidence for antitumor efficacy of the HStk system has also been demonstrated in a rat model of colorectal metastasis to the liver [10]. In this model, BDIX rats were injected under the liver capsule with a syngeneic colon carcinoma cell line. After intratumoral injection of HStk VPC was performed, animals received GCV. Necropsy at the end of GCV treatment (day 15) revealed that approximately one-third of the animals were pathologically tumor free. These preclinical findings demonstrate the efficacy of direct inoculation of a tumor with HStk retroviral VPC to mediate complete tumor destruction.

A number of parameters have been suggested to be important for the observed efficacy of this enzyme/prodrug system. Several determinants, essential for tumor response, have been previously described including gene transfer efficiency, metabolic cooperation, and gene expression level. One of the unique features of the tumor destruction in the HStk system is the observation that not all the tumor cells must contain the inserted gene in order to be killed by GCV [1]. When tumor cell cultures containing 50% HStk gene-modified cells mixed with 50% wild-type unmodified tumor cells were treated with GCV, the entire culture stopped proliferating [9,15]. Even in situations in which the mixed tumor contained 90% unmodified, wild-type tumor cells mixed with 10% HStk modified tumor cells, tumor cell proliferation decreased 70–100% compared to controls. The mechanism of this apparent metabolic cooperation phenomenon is not yet completely understood. In cell culture experiments using radiolabeled GCV, GCV or its metabolites passed from HStk-positive to HStk-negative cells and mediated toxicity only under conditions in which the HStk-positive and -negative cells are in contact with each other [13,14]. The current working hypothesis is that gap junctions allow the passage of the GCV metabolites between tumor cells, resulting in inhibition of DNA synthesis and death of adjacent tumor cells. Furthermore, evidence suggests that the effects of this enzyme and prodrug system require an intact immune system for the best responses. For example, in an intraperitoneal model, immunocompetent animals injected with colonic adenocarcinoma could be rendered pathologically disease free, while athymic nude mice injected with ovarian carcinoma demonstrated prolonged survival but were not rendered pathologically disease free [7]. The significance of an intact immune system was also noted in a study combining the effects of the HStk and interleukin-2 (IL-2) genes [21]. Another group demonstrated that the coinjection of the MC26 mouse colon carcinoma cell line and HStk VPC followed by GCV resulted in almost total tumor regression in the immunocompetent BALB/c mice, but not in athymic BALB/c mice [22]. Thus, the destruction of tumors by this enzyme and prodrug systems can be enhanced by an intact immune system in vivo.

Several groups have developed the use of tumor-

TABLE II. In Vivo Tumor Responses in BALB/c Mice Bearing Subcutaneous CT-26 Adenocarcinoma Tumors With Varying Mixtures of HStk-Transduced or No Vector Cells*

Group	Nontransduced CT-26 cells	Treatment cells used in mixture
A	1×10^5	None
B	1×10^5	1×10^5 CT-26 TK
C	1×10^5	1×10^4 CT-26 TK
D	None	1×10^5 CT-26 TK

*Complete responses are reported for animals that had complete resolution of visible subcutaneous tumor from the 5 animals in the group.

specific promoters to target the HStk system. One group used tumor-specific promoters for melanocytic cells to target gene expression [11]. These gene constructs showed high levels of specific expression in human and murine melanoma cell lines and only basal levels of activity in a range of other cell types. Another group has joined the human surfactant protein A (SPA) transcriptional regulatory elements to the HStk-coding region [23]. Gene transfer into non-small cell lung cancer (NSCLC) cell lines in vitro resulted in selective destruction of cells expressing SPA after treatment with GCV. Tissue-specific promoters are also under development for colon cancer targeting. The carcinoembryonic antigen (CEA) promoter has been demonstrated to be able to target therapeutic gene expression into colon carcinomas [24]. This promoter has been well characterized [25] and also functions in other tumors such as lung cancer that express the CEA [26].

In our studies, we considered the in vivo cooperative killing effect of transduced colon cancer cells on nontransduced tumor cells. The in vivo effects of the system demonstrate a close similarity to the in vitro observations. The contribution of the immune system in vivo has been investigated and seems to be an important contributing component, but its overall contribution has not yet been established [20]. Although transduced tumor cells are exquisitely sensitive to GCV in vitro, animals need to receive a sufficient dose to allow adequate drug concentrations at the tumor site. The usefulness of this enzyme and prodrug system in human clinical trials will be dependent on the efficiency of gene transfer, the ability to stimulate the immune system, and achieving effective doses of GCV at the tumor site. Problems that might be anticipated include the development of GCV resistance in transduced tumor cells, poor cooperative killing effects between cells of certain tumors, or a high fraction of the tumor being in phase G_0 of the cell cycle. Each of these problems may be addressed in the future designs of enzyme and prodrug strategies.

ACKNOWLEDGMENTS

We thank Jamie Stratton for technical assistance.

REFERENCES

1. Moolten FL: Tumor chemosensitivity conferred by inserted herpes thymidine kinase genes: Paradigm for a prospective cancer control strategy. Cancer Res 1986;46:5276–5281.
2. Moolten FL: Mosaicism induced by gene insertion as a means of improving chemotherapeutic selectivity. Crit Rev Immunol 1990;10:203–233.
3. Heyman RA, Borrelli E, Lesley J, et al.: Thymidine kinase obliteration: Creation of transgenic mice with controlled immune deficiency. Proc Natl Acad Sci USA 1989;86:2698–2702.
4. Ezzeddine ZD, Martuza RL, Platika D, et al.: Selective killing of glioma cells in culture and in vivo by retrovirus transfer of the herpes simplex virus thymidine kinase gene. New Biol 1991;3:608–614.
5. Moolten FL, Wells JM: Curability of tumors bearing herpes thymidine kinase genes transferred by retroviral vectors. J Natl Cancer Inst 1990;82:297–300.
6. Oldfield EH, Ram Z, Culver KW, et al.: Gene therapy for brain tumors using intra-tumoral transduction with the thymidine kinase gene and intravenous ganciclovir. Hum Gene Ther 1993;4:39–69.
7. Link CJ, Moorman D, Seregina T, et al.: A phase I trial of in vivo gene therapy with the Herpes simplex thymidine kinase/ganciclovir system for the treatment of refractory or recurrent ovarian cancer. Hum Gene Ther 1996;7:1161–1179.
8. Short MP, Choi BC, Lee JK, et al.: Gene delivery to glioma cells in rat brain by grafting of a retrovirus packaging cell line. J Neurosci Res 1990;27:427–439.
9. Culver KW, Ram Z, Walbridge S, et al.: In vivo gene transfer with retroviral vector-producer cells for treatment of experimental brain tumors. Science 1992;256:1550–1552.
10. Caruso M, Panis Y, Gagandeep S, et al.: Regression of established macroscopic liver metastases after in situ transduction of a suicide gene. Proc Natl Acad Sci USA 1993;90:7024–7028.
11. Vile RG, Hart IR: In vitro and in vivo targeting of gene expression to melanoma cells. Cancer Res 1993;53:962–967.
12. Chen SH, Shine HD, Goodman JC, Woo SL: Gene therapy for brain tumors: regression of experimental gliomas by adenovirus-mediated gene transfer in vivo. Proc Natl Acad Sci USA 1994;91:3054–3057.
13. Freeman SM, Abboud CN, Whartenby KA, et al.: The bystander effect: tumor regression when a fraction of the tumor mass is genetically modified. Cancer Res. 1993;53:5274–5283.
14. Bi WL, Parysek LM, Warnick R, Stambrook PJ: In vitro evidence that metabolic cooperation is responsible for the bystander effect observed with HSV tk retroviral gene therapy. Hum Gen Ther 1993;4:725–731.
15. Link CJ, Kolb E, Muldoon R: Preliminary in vitro efficacy and toxicities studies of the herpes simplex thymidine kinase gene system for the treatment of breast cancer. Hybridoma 1995;14:143–147.
16. Ram Z, Culver KW, Walbridge S, et al.: Toxicity studies of retroviral-mediated gene transfer for the treatment of brain tumors. J Neurosurg 1993;79:400.
17. Moorman DM, Butler DA, Stanley JD, et al.: Survival and toxicity of xenogenic murine retroviral vector producer cells in liver. J Surg Oncol 1994;57:152–156.
18. Miller AD, Rosman GJ: Improved retroviral vectors for gene transfer and expression. Biotechniques 1989;7:980–990.
19. Tapscott SJ, Miller AD, Olson JM, et al.: Gene therapy of rat 9L gliosarcoma tumors by transduction with selectable genes does not require drug selection. Proc Natl Acad Sci USA 1994;91:8185–8189.
20. Ram Z, Culver KW, Walbridge S, et al.: In situ retroviral-mediated gene transfer for the treatment of brain tumors in rats. Cancer Res 1993;53:83–88.
21. Chen S, Li Chen XH, Wang Y, et al.: Combination gene therapy for liver metastasis of colon carcinoma in vivo. Proc Natl Acad Sci USA 1995;92:2577–2581.
22. Gagandeep S, Brew R, Green B, et al.: Prodrug-activated gene therapy: involvement of an immunological component in the ''bystander effect.'' Cancer Gene Ther 1996;3(2):83–88.
23. Smith MJ, Rousculp MD, Goldsmith KT, et al.: Surfactant protein A-directed toxin gene kills lung cancer cells in vitro. Hum Gene Ther 1994;5:29–35.
24. Tanaka T, Kanai F, Okabe S, et al.: Adenovirus-mediated prodrug gene therapy for carcinoembryonic antigen-producing human gastric carcinoma cells in vitro. Cancer Res 1996;56:1341–1345.
25. Schrewe H, Thompson J, Bona M, et al.: Cloning of the complete gene for carcinoembryonic antigen: analysis of its promoter indicates a region conveying cell type-specific expression. Mol Cell Biol 1990;10:2738–2748.
26. Osaki T, Tanio Y, Tachibana I, et al.: Gene therapy for carcinoembryonic antigen-producing human lung cancer cells by cell type-specific expression of herpes simplex virus thymidine kinase gene. Cancer Res 1994;54:5258–5261.